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ORIGINAL ARTICLE

Tetradecylthioacetic Acid Increases Hepatic Mitochondrial β-Oxidation and Alters Fatty Acid Composition in a Mouse Model of Chronic Inflammation

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Abstract The administration of tetradecylthioacetic acid (TTA), a hypolipidemic and anti-inflammatory modified bioactive fatty acid, has in several experiments based on high fat diets been shown to improve lipid transport and utilization. It was suggested that increased mitochondrial and peroxisomal fatty acid oxidation in the liver of Wistar rats results in reduced plasma triacylglycerol (TAG) levels. Here we assessed the potential of TTA to prevent tumor necrosis factor (TNF) α-induced lipid modifications in human TNFα (hTNFα) transgenic mice. These mice are characterized by reduced β-oxidation and changed fatty acid composition in the liver. The effect of dietary treatment with TTA on persistent, low-grade hTNFα overexpression in mice showed a beneficial effect through decreasing TAG plasma concentrations and positively affecting saturated and monounsaturated fatty acid proportions in the liver, leading to an increased anti-inflammatory fatty acid index in this group. We also observed an increase of mitochondrial β-oxidation in the livers of TTA treated mice. Concomitantly, there were enhanced plasma levels of carnitine, acetyl carnitine, propionyl carnitine, and octanoyl carnitine, no changed levels in trimethyllysine and palmitoyl carnitine, and a decreased level of the precursor for carnitine, called γ-butyrobetaine. Nevertheless, TTA administration led to increased hepatic TAG levels that warrant further investigations to ascertain that TTA may be a promising candidate for use in the amelioration of inflammatory disorders characterized by changed lipid metabolism due to raised TNFα levels.

Keywords Tetradecylthioacetic acid · hTNFα transgenic mice · Low-grade inflammation · Dietary treatment · Plasma · Liver

Abbreviations

ACC	Acetyl-CoA carboxylase
ACS	Acyl-CoA synthetase
CPT-I and -II	Carnitine palmitoyltransferase-I and -II
FAO	Fatty acyl-CoA oxidase
FAS	Fatty acid synthase
HDL	High-density lipoprotein
HMG-CoA synthase	3-Hydroxy-3-methylglutaryl-coenzyme A synthase
HPLC	High-performance liquid chromatography
MUFA	Monounsaturated fatty acid(s)
PPAR	Peroxisome proliferator-activated receptor(s)
PUFA	Polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)
TAG	Triacylglycerol
TCA	Tricarboxylic acid cycle (Krebs cycle)
hTNFα	Human tumor necrosis factor α
TTA	Tetradecylthioacetic acid
VLDL	Very low density lipoprotein

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Introduction

Chronic low-grade inflammation accompanies the development of metabolic syndrome features, like abdominal obesity, dyslipidemia, hypertension, and insulin resistance, known risk factors for cardiovascular disease affecting millions of people worldwide. The first indication that there is a connection between obesity, diabetes and chronic inflammation came from the observation that the pro-inflammatory cytokine tumor necrosis factor (TNF) α is overexpressed in adipose tissues of obese mice and humans [1, 2]. Moreover, its inactivation with anti-TNFα antibodies improved insulin resistance in obese mice [2], whereas TNFα null mice would not develop insulin resistance after diet-induced obesity [3]. The multifunctional cytokine TNFα was shown thereafter to perturb lipid metabolism by increasing free fatty acid production [4, 5], promoting lipolysis [6–9], affecting lipid-metabolism-related gene expression [10, 11], controlling cholesterol metabolism [12, 13], and influencing expression and secretion of other adipokines [14–17].

Used as a model for persistent low-grade TNFα exposure, we have found previously that hTNFα transgenic mice show a down-regulation of peroxisome proliferator-activated receptor (PPAR) α target genes [18]. The mitochondrial enzymes involved in hepatic lipid metabolism were influenced, leading to changes in fatty acid synthesis and oxidation [18]. In particular, not only carnitine palmitoyltransferase-I and -II (CPT-I and -II), proteins important for the β-oxidation of long-chain fatty acids in mitochondria, but also fatty acyl-CoA oxidase (FAO), which is important for peroxisomal β-oxidation, proved to have decreased hepatic activity in the hTNFα transgenic mice [18]. In addition, TNFα overexpression was associated with a significant reduction of hepatic mRNA levels of mitochondrial HMG-CoA synthase, the rate-limiting enzyme in ketogenesis [18]. Moreover, lipogenesis is affected by TNFα. Namely, the two important enzymes in lipogenesis, acetyl-CoA carboxylase (ACC), which produces malonyl-CoA for fatty acid synthesis, showed a tendency for lowered activity, whereas fatty acid synthase (FAS), an enzyme involved in long-term regulation of fatty acid synthesis, displayed a significantly decreased activity [18].

As lipids have the ability to modulate metabolic, inflammatory and innate immune processes [19], we investigated in the present study, whether the fatty acid analogue tetradecylthioacetic acid (TTA) could counteract the health risks as a consequence of TNFα-overexpression. To be able to investigate inflammation in relation to lipid accumulation as seen in the nutritional disorder of obesity, we chose to administer a high-fat diet. TTA is a saturated fatty acid containing 16 carbons and one sulfur atom at position three of the carbon chain from the alpha end, a characteristic that results in its increased metabolic stability [20]. TTA is known to act, at least partly, through the activation of PPAR [21–23], and influence plasma lipids by increasing hepatic β-oxidation [24, 25]. Moreover, we have reported that TTA has antioxidant and antiinflammatory properties [26].

In the present work, we show that the administration of TTA to hTNFα transgenic mice fed a high-fat diet showed beneficial effects on serum cholesterol and triacylglycerol (TAG) levels, hepatic fatty acid composition and cholesterol levels, with a concurrent increase in hepatic β-oxidation and fatty inflammatory index.

Experimental Procedure

Transgenic Mice

The study was performed on female transgenic mice expressing low levels of human TNFα (hTNFα) mice from Taconic (Germantown, USA). The transgenic mouse line was generated in strain C57Bl/6 [27]. The experiments were performed in accordance with, and under the approval of, the Norwegian State Board for Biological Experiments, the Guide for the Care and Use of Laboratory Animals, and the Guidelines of the Animal Welfare Act. The mice were between 6 and 8 weeks of age at the start of the experimental feeding and were divided into two experimental groups of five animals each with comparable mean body weight. They were housed in cages with constant temperature (22 ± 2 °C) and humidity (55 ± 5%), where they were exposed to a 12 h light-dark cycle (light from 07.00 to 19.00) and had unrestricted access to tap water and food. The mice were acclimatized to these conditions before the start of the experiment.

Protein and fat of the feeding diets were from casein sodium salt from bovine milk, 20% (Sigma-Aldrich Norway AS, Oslo, Norway) and lard, 23% (Ten Kate Vetten BV, Musselkanaal, Netherlands) plus soy oil, 2% (Dyets Inc., Bethlehem, PA, USA). In addition, in the TTA group 0.6% of the lard was substituted by TTA. The TTA was synthesized as described earlier [28]. There were no significant differences in fatty acid composition between control and TTA diets, except the TTA content (SFA 43.9 vs. 42.8 wt%, MUFA 38.8 vs. 37.8 wt%, PUFAn-3 1.51 vs. 1.48 wt%, PUFAn-6 15.6 vs. 15.3 wt%, TTA 0.0 vs 2.5 wt%).

The mice were anaesthetized under fasting conditions by inhalation of 2% isoflurane (Schering-Plough, Kent, UK) after two weeks of feeding. Blood was collected by aortic

puncture with 7.5% EDTA and immediately chilled on ice. Plasma was prepared and stored at −80 °C prior to analysis. Parts of the liver were used for β-oxidation analysis and chilled on ice, and the rest was freeze-clamped and stored at −80 °C until the analysis of fatty acids, triacylglycerols, cholesterol, and enzyme activities.

Plasma and Hepatic Lipids

Liver lipids were extracted according to Bligh and Dyer [29], evaporated under nitrogen, and redissolved in isopropanol before analysis. Lipids were subsequently measured enzymatically on a Hitachi 917 system (Roche Diagnostics GmbH, Mannheim, Germany) using the triacylglycerol (GPO-PAP) and cholesterol kit (CHOD-PAP) from Roche Diagnostics (Mannheim, Germany) and the phospholipid kit from bioMérieux SA (Marcy l'Etoile, France).

Hepatic Fatty Acid and Plasma Carnitine Compositions

Total hepatic fatty acid composition was analyzed as described previously [18]. The anti-inflammatory fatty acid index was calculated as (docosapentaenoic acid + docosahexaenoic acid + dihomo-γ-linolenic acid + eicosapentaenoic acid)x100/arachidonic acid. Slightly different indexes have been used by [30, 31]. Free carnitine, short-, medium-, and long-chain acylcarnitines, and the precursors for carnitine, trimethyllysine and γ-butyrobetaine, respectively, were analysed in plasma using HPLC/MS/MS as described previously [32] with some modifications (Svardal et al., in preparation).

Hepatic Enzyme Activities

The livers were homogenized and fractionated as described earlier [33]. Palmitoyl-CoA oxidation was measured in a mitochondria-enriched extract from liver as acid-soluble products [34]. The activities of carnitine palmitoyltransferase (CPT)-I [35] and acyl-CoA synthetase (ACS) [35] were measured in the mitochondrial fraction. Fatty acid synthase (FAS) was measured in the post-nuclear fraction as described by Skorve et al. [36].

Statistical Analysis

Data sets were analyzed using Prism Software (Graph-Pad Software, San Diego, CA) to generate the figures and determine statistical significance. The results are shown as means with their standard deviations (S.D.). A *t*-test was used to determine significant differences between the control and the TTA treatment group. *P*-values < 0.05 were considered significant.

Results

Body/Liver Weights and Feed Intake

We found that TTA supplementation for two weeks to hTNFα-overexpressing mice promoted a significant decrease in body weights (−0.8 ± 0.8 g) as compared to high-fat-fed control animals (1.4 ± 0.6 g) (Table 1). Whereas liver weights and liver index were significantly increased in the TTA supplemented group (1.4 ± 0.09 and 7.2 ± 0.43) in comparison to the control group (0.9 ± 0.08 and 4.3 ± 0.3). The TTA group displayed a reduced total feed intake, 30 g versus 34 g of diet per TTA- or control-fed mouse, respectively. Thus, the TTA group seems more efficient in converting feed into increased body mass, as shown by a lower total feed efficiency (−0.03) in comparison to the control group (0.04).

Serum and Hepatic Lipids

In previous studies, comparing transgenic hTNFα-overexpressing with wildtype mice, TNFα interfered with lipid metabolism, leading to increased hepatic TAG and total cholesterol levels. Serum cholesterol concentrations were decreased, whereas serum TAG levels were unchanged [18].

In this study, two weeks of 0.6% TTA administration promoted positive effects on plasma parameters in hTNFα-overexpressing mice fed a high fat diet. The transgenic mice showed increased levels of total cholesterol due to a 39% increase in HDL-cholesterol in the TTA treated group (Fig. 1a) as compared to the high fat-fed controls. With regard to plasma TAG, a drastic reduction of 54% was observed after TTA treatment (Fig. 1b) as compared to the control. Plasma phospholipids (Fig. 1c) and free fatty acids (data not shown) were not significantly changed. The hepatic levels of total cholesterol showed a non-significant tendency to decrease in the TTA-treated group (Fig. 1d), and the already high TAG amounts in transgenic mice

Table 1 Body and liver weights, liver index [100 × (liver weight in g/body weight in g)], total feed intake, and feed efficiency (weight gain in g/food intake in g)

	Control	TTA
Initial body weight (g)	19.2 ± 1.1	20.2 ± 1.3
Final body weight (g)	20.6 ± 1.5	19.4 ± 1.3
Body weight gain (g)	1.4 ± 0.6	−0.8 ± 0.8
Liver weight (g)	0.9 ± 0.08	1.4 ± 0.09
Liver index	4.3 ± 0.3	7.2 ± 0.43
Total feed intake (g)	34.2	30
Total feed efficiency	0.04	−0.03

Values are mean ± S.D. ($n = 5$)

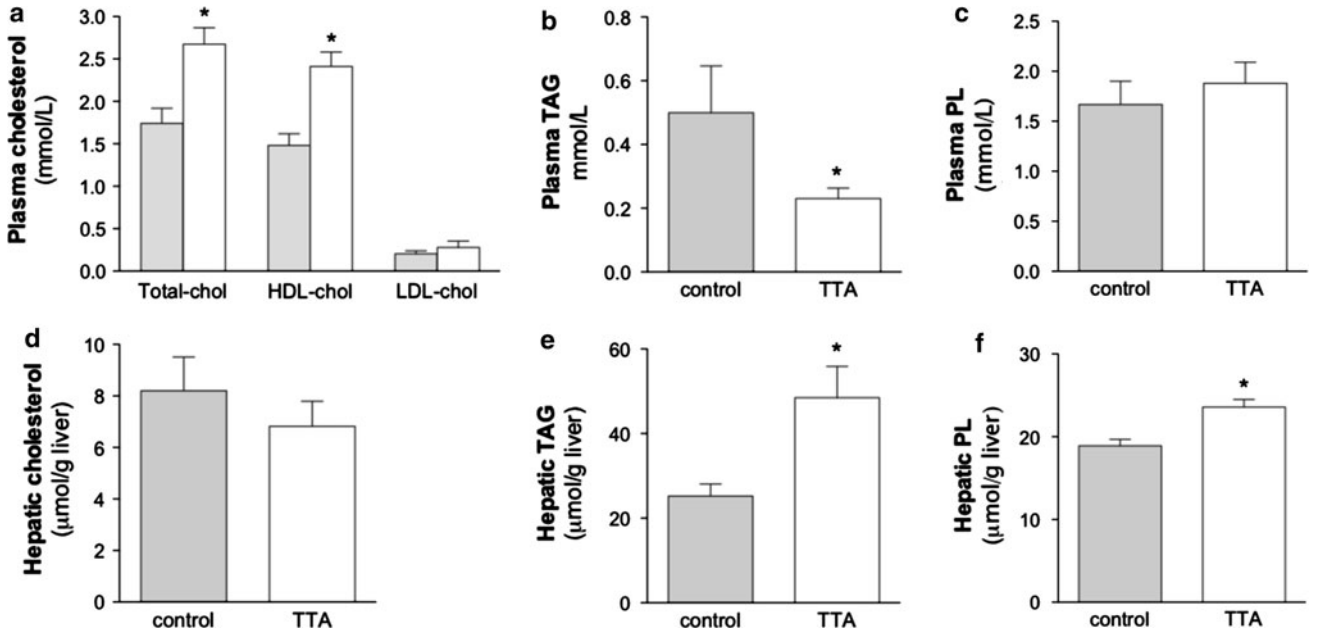

Fig. 1 TTA treatment induces a significant increase in plasma HDL-cholesterol levels in hTNFα transgenic mice (**a**) and a decrease in plasma TAG levels (**b**), whereas phospholipids showed no change (**c**). Hepatic cholesterol showed no significant change (**d**), but a TAG and phospholipid-increasing effect in the liver was found after treatment with TTA (**e**, **f**). Data are means ± S.D. ($n = 5$). * denotes statistical significant differences by Student's *t*-test between control (*grey bars*) and TTA (*white bars*) ($P < 0.05$). *chol*, cholesterol; *HDL*, high-density lipoprotein; *LDL*, low density lipoprotein; *PL*, phospholipid; *AG*, triacylglycerol; *TTA*, tetradecylthioacetic acid

further increased after TTA treatment (Fig. 1e). The hepatic phospholipid level was also elevated after TTA administration in comparison to control mice (Fig. 1f).

Fatty Acid Composition in Liver

In order to investigate if the results obtained for hepatic lipids were specific for cholesterol and TAG or if fatty acids were also affected significantly, we performed an analysis of the total hepatic fatty acid composition. We have previously demonstrated an increased weight % of saturated fatty acids (SFAs) in TNFα-overexpressing mice on a chow diet [18].

Here we observed a comparable level of SFAs in TNFα-overexpressing mice (in spite of higher dietary SFA content in the high-fat diet used- see Discussion). TTA treatment lead to decreased weight % in saturated fatty acid levels (Fig. 2a). The most significant individual decreases could be found in the fatty acids C15:0, C17:0, C18:0, C22:0, C23:0, and C24:0 (Table 2). The most impact on decreasing total SFAs had C18:0.

The relative amounts of monounsaturated fatty acids (MUFA) that were shown to be decreased in hTNFα transgenic in comparison to wildtype mice [18], were significantly increased by TTA in the diet (Fig. 2b). This was mainly due to the increased amount of oleic acid (C18:1n-9) that was to some extent induced by increased Δ9 desaturase activity as the index Δ9 desaturase activity suggests (Table 2).

A significant decrease in weight % of n-3 and n-6 polyunsaturated fatty acids (PUFA) was observed in the transgenic mice treated with TTA (Fig. 2c and d) when compared to control animals. This was reflected in decreased amounts of the n-3 PUFA alpha-linolenic acid (18:3n-3), stearidonic acid (C18:4n-3) and docosahexaenoic acid (C22:6n-3), along with linoleic acid (C18:2n-6) and arachidonic acid (C20:4n-6) that can mainly be attributed to lowered levels of n-6 PUFA (Table 1). The sums result in a decreased ratio of n-3 to n-6 PUFA (Table 1). TTA seems to counteract the increased PUFA levels seen in transgenic mice in contrast to wildtype mice that were due to increased weight % of n-3, with unchanged n-6 levels, giving an increased n-3 to n-6 ratio [18].

In addition, after TTA administration, there was a strong decrease in the C20:4n-6/C20:3n-6 ratio as an indirect measure of the n-6 Δ5 desaturase activity, an enzyme important for the production of arachidonic acid (Table 2). This indicates that TTA might decrease the hepatic desaturation of dihomo-γ-linolenic acid (20:3n-6) to arachidonic acid (20:4n-6) in hTNFα transgenic mice. Whereas beforehand, we observed increased ratios pointing towards increased activities of Δ6 and Δ5 desaturases in transgenic mice in comparison to control animals [18].

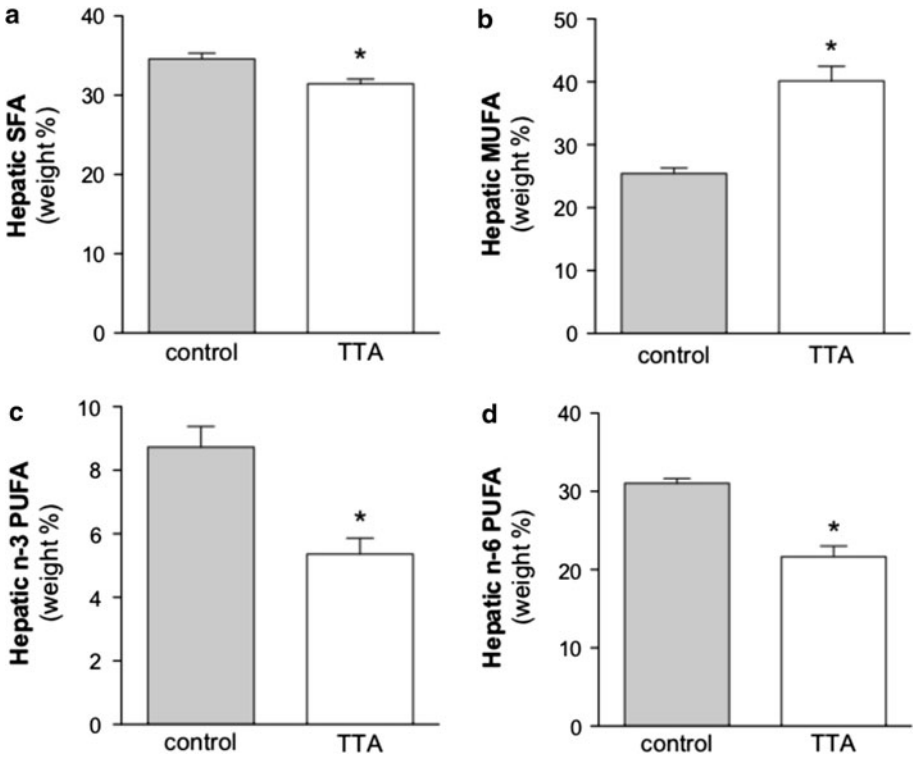

Fig. 2 Dietary treatment for 2 weeks with TTA affects hepatic fatty acid composition and changes saturated fatty acids (**a**), monounsaturated fatty acids (**b**), n-3 polyunsaturated fatty acids (**c**), and n-6 polyunsaturated fatty acids (**d**) levels significantly. The data is given as weight % of total fatty acid ± S.D. ($n = 5$). * denotes statistical significant differences to the control by Student's t test ($P < 0.05$). *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *TTA* tetradecylthioacetic acid

The anti-inflammatory fatty acid index, calculated as (docosapentaenoic acid + docosahexaenoic acid + dihomo-γ-linolenic acid + eicosapentaenoic acid) × 100/arachidonic acid [30], increased after TTA treatment.

Hepatic β-Oxidation of Fatty Acids

The TAG-lowering effect observed in plasma of hTNAα transgenic mice after TTA treatment, could be due to an enhanced mitochondrial and peroxisomal β-oxidation that is responsible for the shortening of long-chain fatty acyl-CoAs. Indeed, β-oxidation increased by 48% in the TTA treated mice, when using ^{14}C-palmitoyl-CoA as substrate (Fig. 3a), counterbalancing the decreased β-oxidation values from transgenic mice [18]. In the previous study, the sensitivity towards malonyl-CoA, an inhibitor of CPT-I, was unaffected by TNFα [18]. However, in the TTA treated group we detected a lowering of the inhibition capacity of malonyl-CoA from 35% to 14% inhibition (Fig. 3b) and the transport of fatty acids across the mitochondrial outer membrane is facilitated even in the presence of malonyl-CoA.

Activities of Enzymes Involved in Degradation and Biosynthesis of Fatty Acids

In order to explain the mechanisms of TTA in more detail, enzyme activities were measured in the post-nuclear fraction of the liver. The enzymatic activity of CPT-I, the rate-limiting enzyme of mitochondrial β-oxidation, involved in acyl group transport into the matrix, was increased after TTA treatment (Fig. 4a). Likewise, the mitochondrial enzyme acyl-CoA synthetase (ACS) that activates fatty acids prior to oxidation, showed increased activity (Fig. 4b). On the other hand, a major cytosolic, multifunctional enzyme involved in the biosynthesis of fatty acids, called fatty acid synthase (FAS), showed reduced activity (Fig. 4c).

Oxidation of fatty acids can be facilitated not only by the up-regulation of CPT-I, but also by an increased concentration of its substrate carnitine, to ensure efficient transport of fatty acids into mitochondria. Carnitine can be taken up from dietary sources or is endogenously synthesized primarily in the liver and kidney [37, 38]. All other tissues have to take carnitine actively up from the blood. We found that in plasma, free carnitine concentrations have increased during TTA treatment by 36% in comparison to control animals (Fig. 5a). The precursors for carnitine, were either unchanged (trimethyllysine, Fig. 5b) or reduced (γ-butyrobetaine, Fig. 5c). The two quantitatively most important acyl-esters, acetyl carnitine (Fig. 5d) and propionyl carnitine (Fig. 5e) were significantly increased by TTA compared to high fat-fed controls. The plasma medium-chain acylcarnitine, octanoyl carnitine, was significantly increased (Fig. 5f) and the long-chain acylcarnitine, palmitoyl carnitine, remained unchanged by TTA treatment (Fig. 5g).

Table 2 Comparison of hepatic fatty acid composition in control and TTA treated hTNFα transgenic mice

Fatty acid	control	TTA	Level of significance
SFA			
C15:0	0.07 ± 0.002	0.03 ± 0.001	$P < 0.0001$
C16:0	21.42 ± 0.35	24.3 ± 0.204	$P < 0.0001$
C17:0	0.19 ± 0.012	0.10 ± 0.009	$P < 0.0001$
C18:0	12.02 ± 0.554	6.42 ± 0.585	$P < 0.0001$
C22:0	0.15 ± 0.017	0.07 ± 0.016	$P < 0.0001$
C23:0	0.08 ± 0.004	0.03 ± 0.004	$P < 0.0001$
C24:0	0.11 ± 0.009	0.05 ± 0.006	$P < 0.0001$
MUFA			
C16:1	0.02 ± 0.003	0.01 ± 0.001	$P < 0.002$
C16:1n-7	1.03 ± 0.127	1.44 ± 0.291	$P < 0.03$
C18:1n-7	1.6 ± 0.079	1.72 ± 0.23	NS
C18:1n-9	21.77 ± 0.748	35.22 ± 1.796	$P < 0.0001$
C20:1n-9	0.26 ± 0.022	0.49 ± 0.064	$P < 0.0001$
n-6 PUFA			
C18:2n-6	16 ± 0.540	9.90 ± 0.743	$P < 0.0001$
C20:4n-6	12.96 ± 0.788	8.61 ± 0.628	$P < 0.0001$
C20:3n-6	0.9 ± 0.059	2.51 ± 0.291	$P < 0.0001$
n-3 PUFA			
C18:3n-3	0.42 ± 0.027	0.13 ± 0.010	$P < 0.0001$
C18:4n-3	0.04 ± 0.005	0.01 ± 0.002	$P < 0.0001$
C22:6n-3	7.60 ± 0.646	4.49 ± 0.431	$P < 0.0001$
C20:5n-3	0.29 ± 0.028	0.27 ± 0.043	NS
Anti-inflammatory index	70.12 ± 2.883	88.94 ± 4.551	$P < 0.0002$
C20:4n-6/C20:3n-6	14.41 ± 1.429	3.46 ± 0.380	$P < 0.0001$
C18:1n-9/C18:0	1.81 ± 0.117	5.54 ± 0.752	$P < 0.0001$

The anti-inflammatory index is calculated as described in Experimental Procedure. The ratio of C20:4n-6 to C20:3n-6 gives an indirect index of the n-6 Δ5 desaturase activity. The data are given as weight % ± S.D. ($n = 5$). A two sample, two-tailed *t*-test assuming equal variance was calculated. NS means not significant

Discussion

Obesity-related metabolic disorders are associated with a state of chronic low-intensity inflammation. Inflammation induces a wide array of metabolic changes in the body and affects expression of many proteins involved in lipid metabolism. The different subtypes of the nuclear ligand-dependent transcription factors, PPAR, play key roles in lipid homeostasis. They are involved in the regulation of lipoprotein metabolism, fatty acid oxidation, glucose and carnitine homeostasis and also seem to be involved in inflammatory processes [39]. TNFα is one of the factors that mediate alterations in lipid metabolism, including decreased PPARα mRNA and protein levels [40]. With the knowledge in mind that previous studies have shown that the biological response to TTA treatment is at least partly a result of PPAR activation, and that this bioactive fatty acid analogue has the ability to activate all three isoforms of PPAR [21, 23, 41], as well as increase the mRNA level of PPARα [42], we wanted to test if TTA was able to counteract TNFα-induced metabolic aberrations.

In the present study we demonstrated that TTA, in addition to its health-promoting effects in previous in vivo [43] and in vitro experiments [26, 44], could also ameliorate several parameters in a mouse model of chronic inflammation. TNFα might induce hypertriglyceridemia, characterized by the accumulation of very low density lipoprotein (VLDL) in the plasma, due to impaired removal of VLDL [45] and increased hepatic lipogenesis [46]. TAG-rich lipoproteins were shown to be important for the acute phase response by binding to endotoxins to reduce their harmful action [47]. However, systemic low-grade inflammation with elevated circulating levels of TNFα seen in obese subjects is typically associated with high plasma TAG levels that will increase the risk for metabolic and cardiovascular complications [48]. It is therefore of importance to note that TTA has the capability to strongly reduce plasma TAG levels in a chronic inflammatory state with persistently high TNFα levels.

The observation of decreased TAG in the plasma is compatible with the hypothesis that there is a higher flux of fatty acids from the plasma to the liver, with a

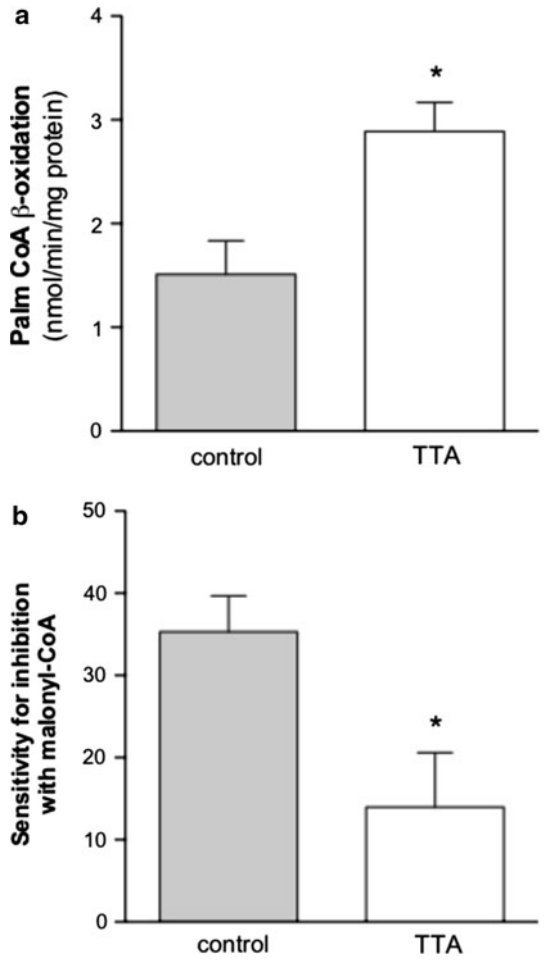

Fig. 3 Palmitoyl-CoA β-oxidation is increased in liver mitochondria of TTA-treated mice (**a**) and the sensitivity for inhibition of oxidation of palmitoyl-CoA with malonyl-CoA (given in % inhibition) is lower in the TTA group compared to high fat-fed controls (**b**). Oxidation of palmitoyl-CoA was measured in purified mitochondria as acid-soluble products. *Bars* indicate $\pm$ S.D. ($n = 5$). * $P < 0.05$ different from the control. *Palm-CoA*, palmitoyl-CoA; *TTA*, tetradecylthioacetic acid

simultaneous reduction in fatty acid biosynthesis. But even though TTA up-regulates hepatic mitochondrial CPT-I and ACS activities, accompanied by an increase in hepatic β-oxidation and plasma carnitine levels, it is not sufficient to deal with the already existing high TAG levels in the transgenic mice. It is noteworthy that TTA has the capability to downregulate the hepatic biosynthesis of fatty acids, since the FAS activity is reduced by 27% in the TTA treated animals. Reduced lipogenesis and increased fatty acid oxidation after TTA treatment seem to be the reason for decreased plasma TAG levels by 54% after TTA treatment. Yet, the exact reasons why hepatic TAG were not affected similarly by TTA are not yet elucidated at the molecular level and will require further detailed studies. It might be that a higher dose of TTA is required to get a more pronounced effect on lipogenesis, thereby influencing hepatic TAG levels. Previously, we have found indications that the effect on hepatic TAG metabolism may be dose dependent and that the amount of hepatic TAG is decreased with increasing doses of TTA [49]. It could also be that a longer feeding period than the two weeks applied is needed to overcome hepatic TAG accumulation. Noteworthy is that we have never detected a TTA-induced liver steatosis in any of the mouse or rat models used previously. It is however of importance to verify in future studies if increasing amounts of TTA could counteract this effect in the mouse model used and if this finding is of significance for humans.

The increased hepatic phospholipid level that we measure in the TTA treated group, is probably a consequence of increased membrane production due to mitochondrial and peroxisomal proliferation as seen after TTA treatment previously [50–52]. Moreover, administration of PPARα ligands has been described to lead to hepatic hypertrophy and hyperplasia leading to increased liver weights as seen in our study. The liver enlargement is however restricted to rodents and has never been described in humans [53–58].

By measuring plasma free carnitine and its acyl-esters, in particular the two most abundant species, acetyl carnitine and propionyl carnitine, we looked at overall changes in the β-oxidation process. We found that plasma free carnitine levels were increased due to TTA administration, as well as the short- and medium-chain carnitine esters, but not long-chain fatty acyl carnitines. It is important to note that in addition to being essential for transport of fatty acids into mitochondria, carnitine is crucial to increase acyl and acetyl group export out of mitochondria into the blood [59]. By increasing carnitine levels through TTA administration, lipotoxicity of β-oxidation metabolites is reduced and mitochondrial capacity is improved. The excess short-chain acyl-esters, that cannot be used by the TCA cycle, will be secreted in urine. The formation of trimethyllysine and its conversion to γ-butyrobetaine is reported to occur in most tissues, but the last step in carnitine biosynthesis, the hydroxylation of γ-butyrobetaine to carnitine, occurs only in liver and kidney in mice. Therefore interorgan transport of γ-butyrobetaine and carnitine are of importance. It was of interest that plasma γ-butyrobetaine levels were decreased, whereas carnitine levels were increased. This may be due to increased consumption of γ-butyrobetaine for carnitine biosynthesis and increased mitochondrial oxidation of long-chain fatty acids.

TTA affects the capacity for insertion of double bonds and counteracts the abnormal hepatic fatty acid composition levels under the influence of TNFα. Correct proportions of fatty acids are crucial to maintain cellular

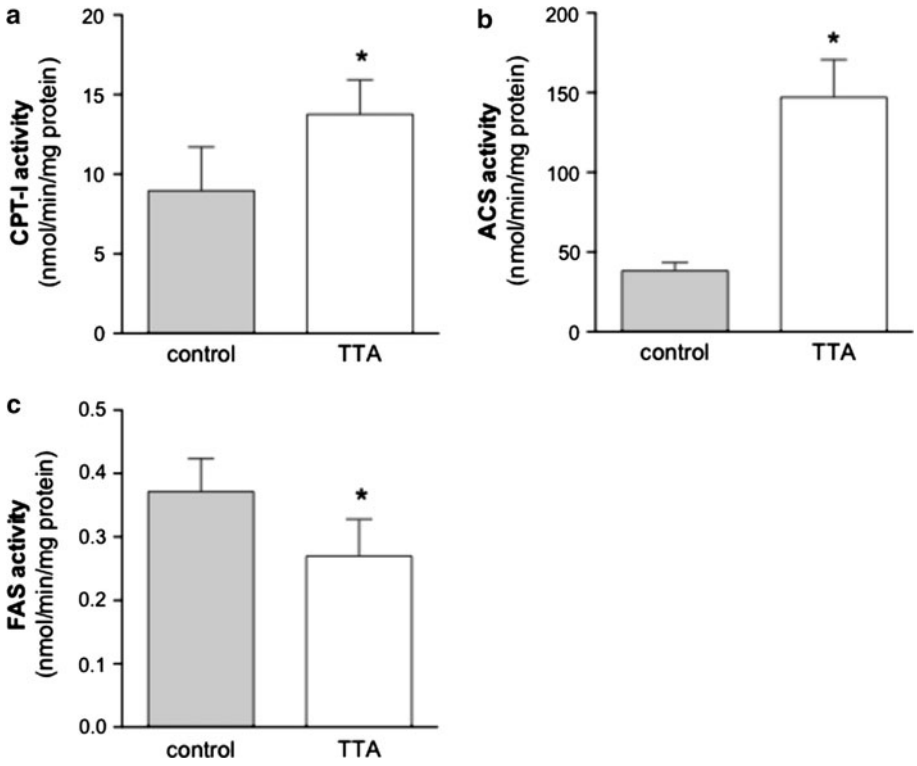

Fig. 4 Enzyme activities of the mitochondrial enzymes CPT-I (**a**) and ACS (**b**) involved in β-oxidation are increased in the liver of hTNFα transgenic mice treated with TTA, whereas the cytosolic FAS activity involved in biosynthesis of fatty acids shows reduced activity after two weeks of treatment (**c**). Values are means ± S.D. ($n = 5$) and * shows a $P < 0.05$ difference from the control. *CPT-I*, carnitine palmitoyl transferase I; *ACS*, acyl-CoA synthetase; *FAS*, fatty acid synthase; *TTA*, tetradecylthioacetic acid

functions, a balance that was disturbed in the TNFα transgenic mice. hTNFα overexpression was accompanied by increased saturated and polyunsaturated fatty acids, but decreased levels of monounsaturated fatty acids in liver [18]. The basic values of these fatty acid families in hTNFα control group in our high fat diet experiment compared to the previous study were less pronounced, which could be partially attributed to a different diet in [18] (low fat standard mouse chow with 26 % SFA, 25 % MUFA, 4 % PUFAn-3 and 45 % PUFAn-6). Nevertheless, it did not affect the trends of fatty acid changes inside the dietary groups (high fat diet and standard chow diet, respectively). Moreover, TTA reduced the n-6 Δ5 desaturase activity index in transgenic mice as fatty acid saturation was increased. This was accompanied by decreases of n-3 and n-6 polyunsaturated fatty acids including stearidonic acid (C18:4n-3) and docosahexaenoic acid (C22:6n-3), as well as linoleic acid (C18:2n-6) and arachidonic acid (C20:4n-6). The amount of arachidonic acid is of importance for the calculation of the anti-inflammatory fatty acid index, and decreased amounts after TTA treatment increased the fatty anti-inflammatory index in this group. Saturated fatty acids, that have pro-inflammatory and insulin-antagonizing properties [60], were decreased by TTA, thereby lowering the risk for the development of metabolic syndrome. On the other hand, monounsaturated fatty acids were increased after TTA treatment. This is potentially beneficial, as high levels were associated with low rates of cardiovascular disease [61]. Another risk factor for cardiovascular disease in humans is low HDL-cholesterol due to its important function in reverse cholesterol transport to the liver [62]. We found elevated plasma HDL-cholesterol levels with an increase of 63% in TTA treated TNFα transgenic mice. However, in a study including a small group of HIV-infected patients, we found that TTA in combination with dietary intervention can reduce total cholesterol, LDL-cholesterol, and LDL/HDL cholesterol, but no increase in HDL-cholesterol could be observed [63]. It has been shown previously that apolipoprotein compositions and responses to PPARα activation are different between humans and rodents [64, 65]. The result of increased HDL-cholesterol in hTNFα overexpressed mice in our study might therefore be rodent-specific. However, effects of PPARα activation on other pathways of lipid metabolism including FA uptake and activation, β-oxidation, and lipogenesis support similarities between mice and humans [39]. This suggests similar responses upon TTA treatment in humans as seen in our mice study.

In summary, given the ability of TTA to increase β-oxidation, reduce plasma TAG, and positively affect hepatic saturated and monounsaturated fatty acid compositions

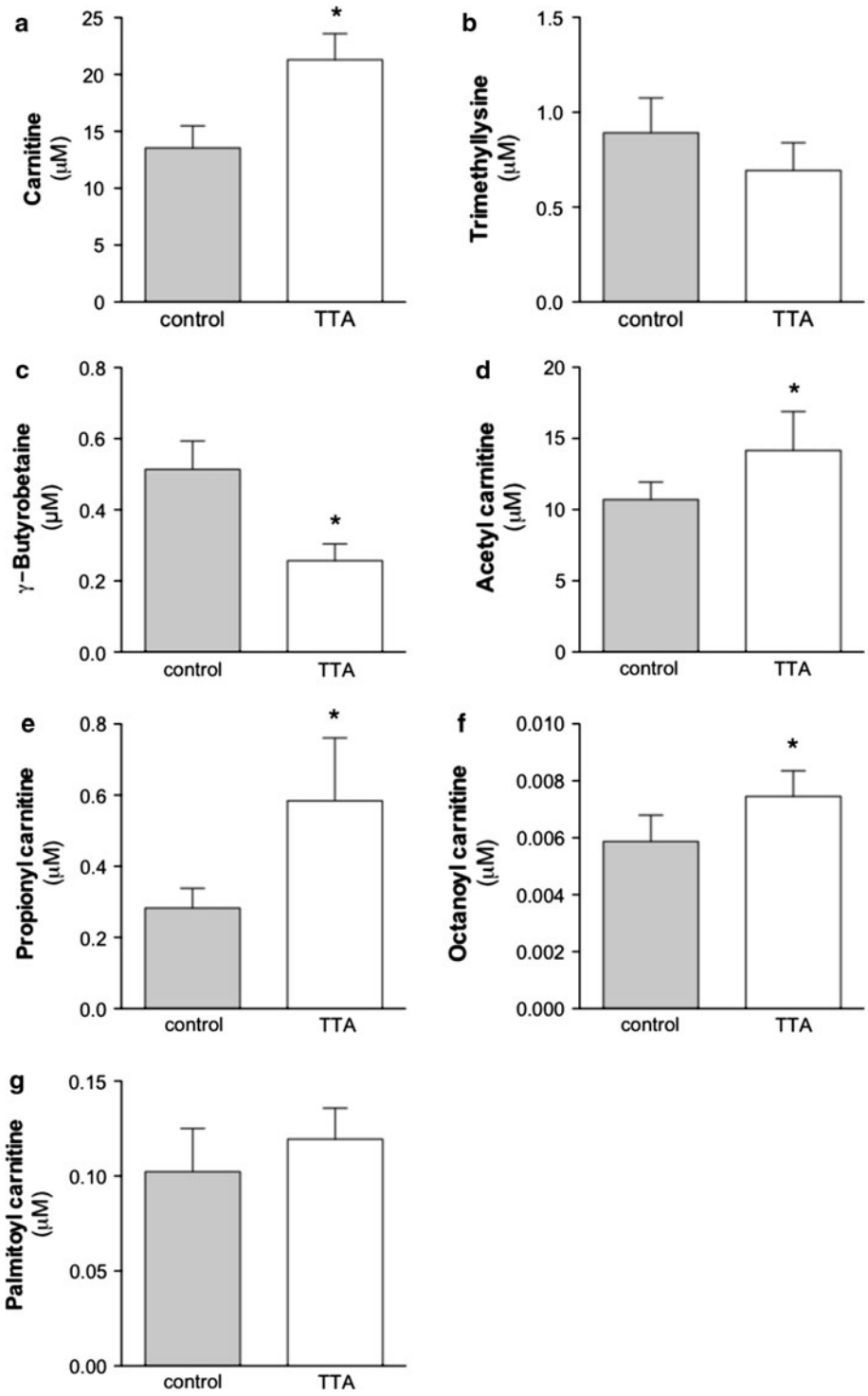

Fig. 5 A change in plasma levels of free carnitine (**a**), trimethyllysine (**b**), γ-butyrobetaine (**c**), acetyl carnitine (**d**), propionyl carnitine (**e**), octanoyl carnitine (**f**), and palmitoyl carnitine (**g**) could be detected after TTA treatment. * denotes statistical significant differences by Student's *t*-test between control (*grey bars*) and TTA (*white bars*) ($P < 0.05$) and *bars* indicate ± S.D. ($n = 5$)

leading to an increase in the anti-inflammatory fatty acid index, indicates that TTA has a high potential to ameliorate chronic inflammation such as with obesity, arthritis or atherosclerosis.

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